

Sub C1

WHAT IS CLAIMED IS:

1. A method of detecting the presence or absence of at least one mismatch between a nucleic acid probe and a nucleic acid target, wherein said probe and target have sequences which differ by not more than five mismatches, said probe comprising a known sequence and a photoactivatable cross-linking agent, which when said probe sequence is hybridized to said target sequence, upon photoactivation forms a covalent bond between said probe sequence and said target sequence, said method comprising:
 - 10 combining, in a hybridizing medium, a nucleic acid sample comprising said target and said probe under mild stringency hybridizing conditions for a time sufficient for said target and said probe to hybridize;
 - 15 irradiating said hybridizing medium to form cross-links between said probe and target sequence to which said probe is hybridized to from cross-linked double-stranded nucleic acid;
 - 20 separating nucleic acid in said hybridizing medium by denaturing electrophoresis and comparing the migratory rate of said cross-linked double-stranded nucleic acid to a known mismatched or matched cross-linked double-stranded nucleic acid standard, whereby the presence or absence of said at least one mismatch is determined.
 2. A method according to Claim 1, wherein said probe is labeled with a detectable label.
 - 25 3. A method according to Claim 1, wherein said sample is prepared using the polymerase chain reaction and said sample nucleic acid is labeled with a detectable label.
 - 30 4. A method according to Claim 1, wherein said electrophoresis is polyacrylamide gel electrophoresis.
 - 35 5. A method of detecting the presence or absence of at least one mismatch between a nucleic acid probe and a nucleic acid target, wherein said probe and target have sequences which differ by not more than five mismatches, said target sequence comprising a nucleic acid molecule of from about 25 to 300 nt and said probe comprising a known sequence of from 15 to 50 nt and a photoactivatable cross-linking agent, which when said probe sequence is hybridized
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to said target sequence, upon photoactivation forms a covalent bond between said probe sequence and said target sequence, said method comprising:

combining, in a hybridizing medium, a nucleic acid sample comprising said target and said probe under mild stringency hybridizing conditions for a time

5 sufficient for said target and said probe to hybridize;

irradiating at a wavelength in the range of about 300 - 400 nm said hybridizing medium to form cross-links between said probe and target sequence to which said probe is hybridized to cross-linked double-stranded nucleic acid;

separating nucleic acid in said hybridizing medium by denaturing

10 electrophoresis and comparing the migratory rate of said cross-linked double-stranded nucleic acid to a known mismatched or matched cross-linked double-stranded nucleic acid standard, whereby the presence or absence of said at least one mismatch is determined.

15 6. A method according to Claim 5, wherein said sample is prepared by restriction enzyme digestion of genomic DNA.

7. A method according to Claim 5, wherein said sample is prepared using the polymerase chain reaction and said sample nucleic acid is labeled with a 20 detectable label.

8. A method according to Claim 5, wherein said probe is labeled with a detectable label.

25 9. A method according to Claim 5, wherein said electrophoresis is polyacrylamide gel electrophoresis.

30 10. A method of detecting the presence or absence of at least one mismatch between a nucleic acid probe and a nucleic acid target, wherein said probe and target have sequences which differ by not more than five mismatches, said target sequence comprising a nucleic acid molecule of from about 25 to 300 nt and said probe comprising a known sequence of from 15 to 50 nt and a photoactivatable cross-linking agent, which when said probe sequence is hybridized to said target sequence, upon photoactivation forms a covalent bond between said probe sequence and said target sequence, said method comprising:

35 combining, in a hybridizing medium, a nucleic acid sample comprising said target and said probe under mild stringency hybridizing conditions equivalent to a

temperature in the range of 25 - 70°C and with 0.1 - 1.5 M sodium for a time sufficient for said target and said probe to hybridize;

- 5 irradiating at a wavelength in the range of about 300 - 400 nm said hybridizing medium to form cross-links between said probe and target sequence to which said probe is hybridized to from cross-linked double-stranded nucleic acid;
- 10 separating nucleic acid in said hybridizing medium by denaturing gel electrophoresis and comparing the migratory rate of said cross-linked double-stranded nucleic acid to a known mismatched or matched cross-linked double-stranded nucleic acid standard, whereby the presence or absence of said at least one mismatch is determined.

11. A method according to Claim 10, wherein said cross-linking agent comprises a coumarinyl group.

- 15 12. A method of detecting the presence or absence of at least one mismatch between a nucleic acid probe and a nucleic acid target, wherein said probe and target have sequences which differ by not more than five mismatches, said target sequence comprising a nucleic acid molecule of from about 25 to 300 nt and said probe comprising a known sequence of from 15 to 50 nt and a
- 20 photoactivatable cross-linking agent, which when said probe sequence is hybridized to said target sequence, upon photoactivation forms a covalent bond between said probe sequence and said target sequence, said method comprising:
- 25 combining, in a hybridizing medium, a nucleic acid sample comprising said target and said probe under high stringency hybridizing conditions for a time sufficient for said target and said probe to hybridize, where a probe complementary to said target results in at least about a 2-fold greater amount of hybridization than a mismatched probe;
- 30 irradiating at a wavelength in the range of about 300 - 400 nm said hybridizing medium to form cross-links between said probe and target sequence to which said probe is hybridized to cross-linked double-stranded nucleic acid;
- 35 separating nucleic acid in said hybridizing medium by denaturing electrophoresis and determining the amount of cross-linked double-stranded nucleic acid, where the amount of cross-linked double-stranded nucleic acid is related to the presence or absence of mismatches between said probe and said target.

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13. A method according to Claim 12, wherein said high stringency conditions are at least equivalent to a temperature in the range of about 40-70° C and 0.05 to 0.5 M sodium ion.

14. A kit comprising two probes, characterized by consisting of from 15 to 50 nt, joined to each of said probes is a photoactivatable cross-linking agent, each of said probes differing with the other probe by not more than 3 mismatches, and being naturally occurring sequences.

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15. A kit according to Claim 14, wherein said photoactivatable cross-linking agent comprises a coumarinyl group.

16. A kit according to Claim 14, wherein said naturally occurring sequences are related by one being the mutant of the other.

10 17. A kit according to Claim 14, wherein said naturally occurring sequences are related by one being the allele of the other.

15 18. A kit according to Claim 14, wherein said probes are labeled with a detectable label.

19. A kit according to Claim 14, wherein each of said probes has a plurality of cross-linking agents.